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Structure, function and evolution of somatic musculature in Dasydytidae (Paucitubulatina, Gastrotricha)

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Abstract The musculature of two species of the gastro-trich taxon Dasydytidae, *Dasydytes* (*Dasydytes*) *goniathrix* and *Haltidytes crassus*, was investigated and described using phalloidin staining, confocal microscopy and computer-aided three-dimensional data analysis. Dasydytidae is a peculiar taxon of freshwater Gastrotricha, containing species that are characterized by different adaptations to a semiplanktonic lifestyle, a rather uncommon feature among primarily benthic Gastrotricha. Like other dasydytid species studied so far, *D. goniathrix* and *H. crassus* possess a system of movable cuticular spines with an associated system of somatic oblique and segmented lateral muscles. The presence of other somatic (dorsodermal muscles R1 and R2) and visceral muscles (musculi ventrales, m. ventrolaterales, m. dorsales, m. helicoidales) known from a wide range of gastrotrich species was confirmed. Regarded from a functional perspective, the earlier proposed antagonistic role of oblique muscles (as spine abductors) and segmented lateral muscles (as adductors) is questioned for the species studied herein. Alternatively, our structural and behavioral observations suggest that muscular spine abduction in *D. goniathrix* is brought about by synergistic contraction of the musculi obliqua and m. laterales, and a passive adduction due to muscle relaxation and elastic recoil of the trunk and cuticle. For *H. crassus*, we hypothesize active muscular spine abduction by contraction of the musculi obliqua plus the last segment of m. laterales accompanied by severe cuticle deformations close to the spine insertions. Adduction is achieved by cuticle reformation due to elasticity and increase in tissue pressure brought about by muscle action, possibly of enforced dorsodermal muscles. The newly obtained and published muscular data of further gastrotrich species were gathered in a species-character matrix. Based on this data set, a maximum parsimony analysis of representatives of the Dasydytidae has been conducted. According to this analysis, there are three well-supported monophyletic lineages within likewise monophyletic Dasydytidae. The first lineage comprises the taxa *Anacanthoderma*, *Stylochaeta* and *Chitonodytes*, the second comprises *Dasydytes*, *Setopus* and *Ornamentula*, and the third represents the taxon *Haltidytes*. Relationships between these clades could be resolved but are only weakly supported. The new phylogenetic hypothesis is used to reconstruct the ancestral character pattern and to infer possible evolutionary transformations within the Dasydytidae.

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Introduction

The application of histochemical and immunohistochemical staining methods and confocal laser scanning microscopy of microscopic taxa has yielded new insights into function, evolution and ontogeny of different organ systems (Wanninger 2007). This is as well the case for the Gastrotricha, a group of exclusive microscopic and aquatic

animals, of which most show a benthic lifestyle. Based on these relatively new techniques, we now possess a good understanding of the architecture of the nervous system of several gastrotrich species (Rothe et al. 2011a, b and references therein). A second organ system that has received considerable attention using these techniques is the muscular system. Until now, the musculature of numerous gastrotrich species of both major clades, the almost exclusive marine Macrodasyida (e.g., Hochberg and Litvaitis 2001a, c, d; Leasi et al. 2006) and the marine- and freshwater-dwelling Paucitubulatina (e.g., Hochberg and Litvaitis 2001b, 2003; Kieneke et al. 2008a; Leasi et al. 2006; Leasi and Todaro 2008, 2009), plus the phylogenetically enigmatic marine taxon *Neodasys* (Hochberg 2005) was studied thoroughly. Based on these data, we now have (1) a better understanding about the organization of the musculature in the last common ancestor of Gastrotricha (see Hochberg 2005) and (2) satisfactory hypotheses about major evolutionary processes in certain lineages like the stepwise modification, reduction and loss of visceral and somatic circular musculature in Paucitubulatina (Leasi and Todaro 2008).

In the stem lineage of the derived freshwater taxon Dasydytidae, there occurred severe evolutionary transformations: (1) transition to a semiplanktonic lifestyle, (2) loss of adhesive organs, (3) expansion of head cilia (rings and tufts) and development of specialized, moveable spines and (4) specialized swimming and predator-escape behaviors. A recent study of the muscular system of two members of the Dasydytidae, *Stylochaeta scirtetica* Brunson, 1950 and *Setopus tongiorgii* (Balsamo, 1983), has revealed a complex system of serially arranged oblique muscles and segmented longitudinal muscles associated with the motile spines. Both muscular components were hypothesized to function as antagonists in the cycle of spine abduction and subsequent adduction (Kieneke et al. 2008a). Furthermore, the study also revealed severe differences between the two species such as a differing number of segments of the longitudinal muscles and oblique muscle pairs or an alternative shape of the oblique components, for example thin muscle strands in *S. tongiorgii* but massive muscle blocks in *S. scirtetica* (Kieneke et al. 2008a). The limited number of species examined from this clade (containing 7 genera; Balsamo et al. 2009) makes evolutionary inference, for example a reconstruction of ancestral character states, highly speculative.

To further increase our knowledge about the evolution of muscular architecture in Gastrotricha, we studied two additional species of Dasydytidae, *Dasydytes* (*Dasydytes*) *goniathrix* Gosse, 1851 and *Haltidytes crassus* (Greuter, 1917). With these data, we present new functional hypotheses on locomotion in Dasydytidae and, when combined with information on the musculature of other gastrotrichs, build a

matrix of muscular characters for examining evolutionary relationships and trends in the Gastrotricha.

Materials and methods

Sampling, preparation and data acquisition

Specimens of *Dasydytes* (*Dasydytes*) *goniathrix* (Dasydytidae, Paucitubulatina) (Fig. 1c) were collected in January 2008 from flooded grassland and wetlands near Veenhusen (East Friesia), northwest Germany (53°17′13″N, 7°30′59″E). Gastrotricha were sampled by using a small plankton net mounted on a stick. Additional specimens for scanning electron microscopy were sampled in October 2008 from an eutrophic ditch in Oldenburg (53°09′45″N, 8°10′41″E) partially covered by floating *Lemna* sp. and containing other submerged plants. Specimens of *H. crassus* (Dasydytidae, Paucitubulatina) were collected in July 2008 from a eutrophic pond in Hohenböcken (Lower Saxony), NW Germany (53°05′28″N, 8°30′08″E), and from the eutrophic ditch in Oldenburg. Gastrotricha were either sampled with a small plankton net or by pouring water through a fine gauze (40 μm mesh size) that was squeezed from submerged vegetation and rotting plant material. We here have to mention that there is some taxonomic confusion regarding species delimitation in two *Haltidytes* species, *H. crassus* and *Haltidytes festinans* (Voigt, 1909). According to Kisielewski (1991), the spine pattern in both species is similar, and the most reliable character for separating them is the considerably different body length in mature specimens, up to 114 μm in *H. festinans* and to 205 μm in *H. crassus*, respectively. Our specimens measured between 120 and 150 μm in total body length and are hence somehow intermediate. Nevertheless, we here assign our specimens to the species *H. crassus*. In a recent faunistic paper on Swedish freshwater Gastrotricha, even smaller specimens measuring between 97 and 130 μm were identified as *H. crassus* (Kånneby 2011). Numeric characters of some of our *Dasydytes* (*Dasydytes*) *goniathrix* specimens (i.e., number of spine groups and number of spines per group) differ from the data provided in the literature even though the spine number is known to be a variable character in that species (Schwank 1990). Furthermore, Schwank (1990) regards *D. goniathrix* and another species of that group—*Dasydytes ornatus* Voigt, 1909—as synonymous. However, we have assigned our specimens to the species *D. goniathrix* due to the presence of unpaired median dorsal spines.

Individual specimens of both species were picked out from the raw samples using a dissecting microscope and a capillary pipette. Live observations and documentation of

Fig. 1 Light microscopy of the investigated species of Dasydytidae (Gastrotricha). **a** *Haltidytes crassus*, habitus. Focal plane on *dorsal side*, **b** focal plane on *ventral side* (both images: brightfield optic). Note the insertions of the ventrolateral trunk spines of group tb, tc, td (*black triangles*). **c** *Dasydytes goniathrix*, habitus. Focal plane on *dorsal side* (differential interference contrast optic). Note the unpaired medial dorsal spines (*asterisk*). *r* rear spines, *ta1-3* paired trunk spines 1-3 of group ta, *tb1* first trunk spine of group tb, *tb2* second trunk spine of group tb, *tc1* first trunk spine of group tc, *tc2* second trunk spine of group tc, *td* trunk spines of group td
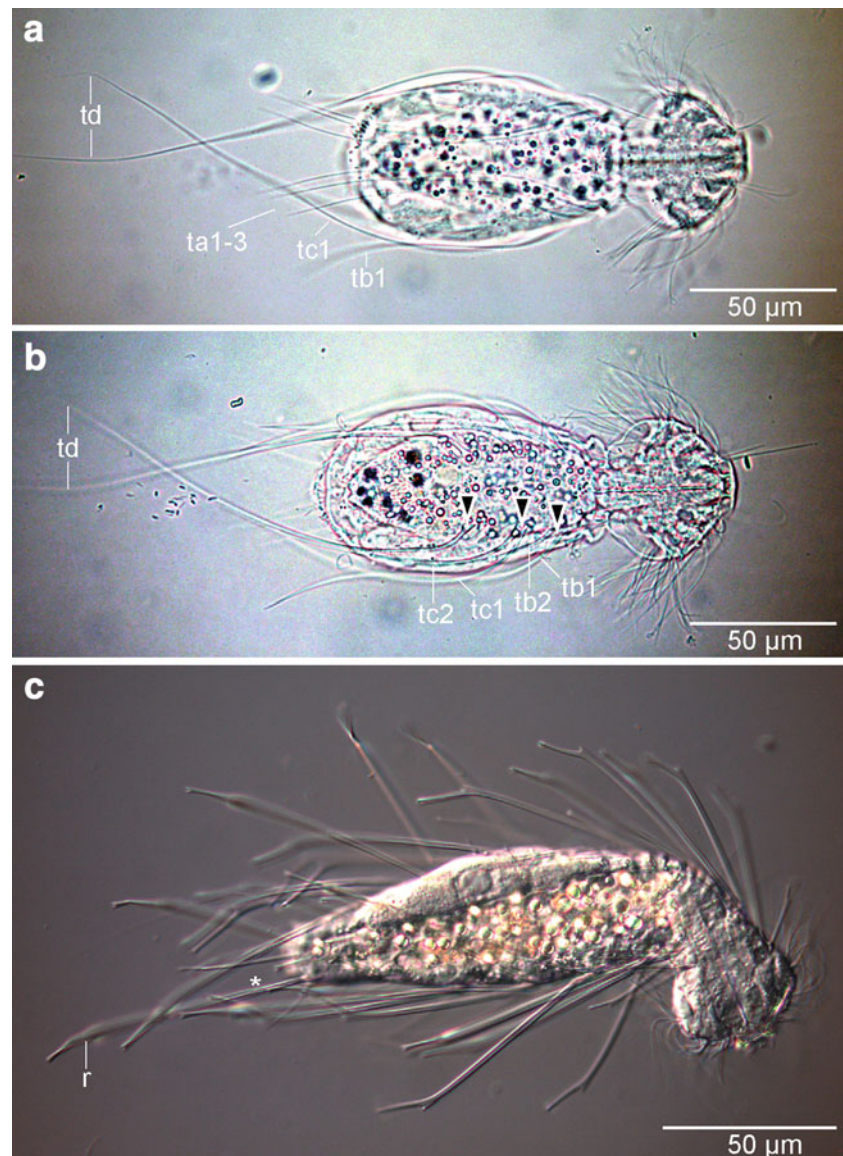
both species were carried out on a Leica DMLB compound microscope equipped with an Olympus Color View I digital camera. For fluorescence studies, animals were first narcotized for few minutes with a 0.25% aqueous solution of bupivacaine (Bucain®, Curasan, Kleinostheim, Germany) and then fixed for 1 h (4°C) with freshly prepared 4% formaldehyde buffered in 0.1 M PBS. After rinsing in 0.1 M PBS, specimens were incubated overnight in a 0.1% solution of Triton X-100 (buffered in 0.1 M PBS, 'permeabilization buffer') to make the integument permeable. For staining, 2 μl of 38 μM methanolic TRITC–phalloidin solution was added to 100 μl of the permeabilization buffer. Specimens were stained herein at 4°C. After 3 h, staining was stopped by rinsing the specimens several times with fresh 0.1 M PBS. Individual specimens were mounted on microscopic slides with Cityfluor® and observed under a

Leica TCS SP 5 confocal laser scanning microscope (DM5000B stand). The excitation wavelength for the fluorochrome (TRITC) was 561 nm, and emission band width has been set to 570–700 nm. We have investigated eight specimens of each species.

For scanning electron microscopic (SEM) investigation, few specimens of both species (*H. crassus*: $N = 3$; *D. goniathrix*: $N = 2$) were fixed with picric acid–formaldehyde (PAF) adjusted to an osmolarity of 239 mOsm (Melone and Ricci 1995). The specimens for SEM observations were dehydrated in an increasing ethanol series (using a minute Teflon container closed by two copper grids usually applied to transmission electron microscopy, constructed by Wilko H. Ahlrichs, University of Oldenburg), critical-point-dried and mounted on a round coverslip coated with a thin layer of special resin (TempFix).

The coverslip was transferred to a standard aluminum SEM stub, and specimens were coated with gold. Observation and documentation were done with a Zeiss DSM 940 scanning electron microscope.

Reconstruction of muscular patterns

Color-coded Z-projections were performed with the Leica LAS AF software. The program ImageJ 1.37v (Abramoff et al. 2004, Rasband 1997–2007) was used to perform Z-projections of subsets of the original image stacks, exported as multiple Tiff files by LAS AF and imported to ImageJ as a single multilayer Tiff image. The main somatic muscular components in both species (muscui laterales, musculi obliqua, dorsodermal muscles) were reconstructed three-dimensionally using the AMIRA 5.0 software package (Visage Imaging^{TM}) and a single representative data set for each species. Digital drawings for this study were created with Adobe Illustrator® CS 5 based on the 3D reconstructions and all available image stacks.

Terminological considerations

It needs to be stressed that, whenever we speak of 'muscles', we are fully aware of the fact that phalloidin staining can only visualize f-actin filaments and not entire muscle cells. For ease of communication, we will nevertheless use the term 'muscle' throughout this paper. The method of phalloidin staining and fluorescence microscopy is furthermore not optimal to resolve the actual insertion of a muscle ending. Nevertheless, when a certain muscle terminates close to the body wall (e.g., the frontal end of the musculi ventrales), we propose an attachment of this muscle to the integument (cuticle plus epidermis) via cell–cell adherence junctions and cell–cuticle hemidesmosomes (Ruppert 1991). In the case of the segmented longitudinal muscles and the serial oblique muscles, an attachment with the bases of the motile spines is assumed.

In the descriptions of the individual muscles, we follow the terminology for musculature of Gastrotricha Paucitubulatina used by Hochberg and Litvaitis (2003). Identical names as in species of the Chaetonotidae (see Hochberg and Litvaitis 2003) and Dasydytidae (see Kieneke et al. 2008a) are assigned to muscles hypothesized homologous among taxa. Criteria for homology used for this assessment are the "Remane criteria": (1) the position and insertions of muscles ("criterion of location") and (2) the different orientations (e.g., longitudinal, circular, oblique muscles), dimensions and shapes of muscles ("criterion of specific quality", see, e.g., Rieger and Tyler 1979, Wägele 2005). In order to provide information on the approximate position of different muscles, we use the percentage body units U (whole-body length = 100%) that is a standard

procedure in gastrotrich taxonomy (e.g., Hummon 1974). For numbering and naming the different groups of motile spines in both investigated species, we use the terminology of Kisielewski (1991). It has to be emphasized that spines that are inserted at the transition from the neck region to the trunk are thus treated as the first group of trunk spines.

Phylogenetic analysis and reconstruction of character evolution

As yet, there is no convincing phylogenetic hypothesis on the internal relationships of the taxon Dasydytidae. In the discussion section, we will briefly report on some shortcomings of the few existing systematizations. In order to study the possible phylogenetic relationships of Dasydytidae and to infer the character evolution, we constructed a species-character matrix using the program NDE 0.5.0 (Page 2001) that operates with the nexus file format (Maddison et al. 1997). The matrix comprises 20 terminal taxa (species), ten of which belonging to the taxon Dasydytidae and the remainder to other taxa of the Gastrotricha Paucitubulatina (see matrix; supplementary file 1). In total, 80 characters with multiple unordered character states were coded, the majority concerning musculature (characters 16–53) and motile spines (characters 54–80, see character and character state description; supplementary file 2). A maximum parsimony analysis was carried out with the program PAUP version 4.0b10 (Swofford 2002). The heuristic search was run with 10.000 replicates (random addition-sequence of taxa) using the TBR algorithm for branch swapping; branches with a length of zero were collapsed to polytomies. *Musellifer delamarei* (Renaud-Mornant, 1968) was used to root the trees. A 50% majority rule consensus tree was calculated from the retained most parsimonious trees. Subsequently, a bootstrap analysis was carried out in order to assess robustness of the resolved clades (2.000 bootstrap replicates, 100 h-search replicates per bootstrap replicate, branch swapping by TBR, polytomization of zero-length branches).

We traced the evolution of character states on the consensus tree topology with the Mesquite software package version 2.75 (Madison and Madison 2011) using the 'trace character history' module. Unambiguous transformations are regarded as possible autapomorphies of the corresponding clade. The 'trace all characters' module was used to reconstruct the character vector of the common ancestor (hypothetical stem species) of Dasydytidae. Different characters within the matrix are logically dependent on others, for example, coding the shape (=character variable) of a structure is dependent on its existence. Hence, we had to code inapplicable character states (–) in different instances that are somehow problematic since the logical dependence between the structure and its variable is not

fully accounted for. Nevertheless, coding of inapplicable character states is preferred here because it saves important phylogenetic information (Lee and Bryant 1999). In terms of character optimization, we have to refer to the fact that all available computer algorithms treat inapplicable character states as missing states (?), which can lead to erroneous reconstructions (Strong and Lipscomb 1999). We therefore checked each optimization result of a given node carefully by hand.

Results

Spination and locomotion

Haltidytes crassus possesses four groups of long and strongly curved trunk spines (*ta–td*) that end in a pointed apex but do not have additional denticles. There are three spines in the first group (*ta1-3*), two in the second and third groups (*tb1-2*, *tc1-2*) and a single spine per side in the fourth group (*td*) of ventrolaterally inserting motile trunk spines (Figs. 1a, b, 3a, b). In their resting position, all spines of the first group and the anterior spines of the second and third groups (*tb1* and *tc1*) continue along the dorsal side of the trunk region, while the posterior spines of the second and third groups (*tb2* and *tc2*) and the paired spine of the fourth group (*td*) extend along the ventrolateral flanks of the trunk. The single pair of spine group 4 is the longest (measuring almost $1.5\times$ the total body length), and both spines cross behind the rounded caudal trunk end.

The animals are adapted to the pelagic life and are smooth swimmers, for which the cilia on the head and on the ventral side of the trunk are utilized (Figs. 1a, b; 3a, b). When the direction of swimming is changed, the head is slightly tilted. When observing specimens under the dissecting microscope using a magnification of $63\times$, it seems that the spines of the first group (*ta*) are lifted a little bit during change in direction. If disturbed, *H. crassus* is able to abduct all motile spines abruptly and very fast (in a fraction of a second) and hence to perform little "jumps" in the water column in order to escape from potential foragers. However, we were neither able to clarify whether these saltatory movements propel the animal backwards as should be expected from spine abduction nor able to clarify whether there are also tumbling movements due to the abduction of spines of only one side.

Dasydytes (*Dasydytes*) *goniathrix* has laterally to ventrolaterally inserting paired groups of motile spines at the head, neck, trunk and rear trunk regions (Figs. 1c; 2a, b; 3c, d). There are three paired groups of head and neck spines (*ca–cc*), six groups of trunk spines (*ta–tf*) and a single pair of rear trunk spines. One specimen of *D. goniathrix* studied by scanning electron microscopy most likely has two, one
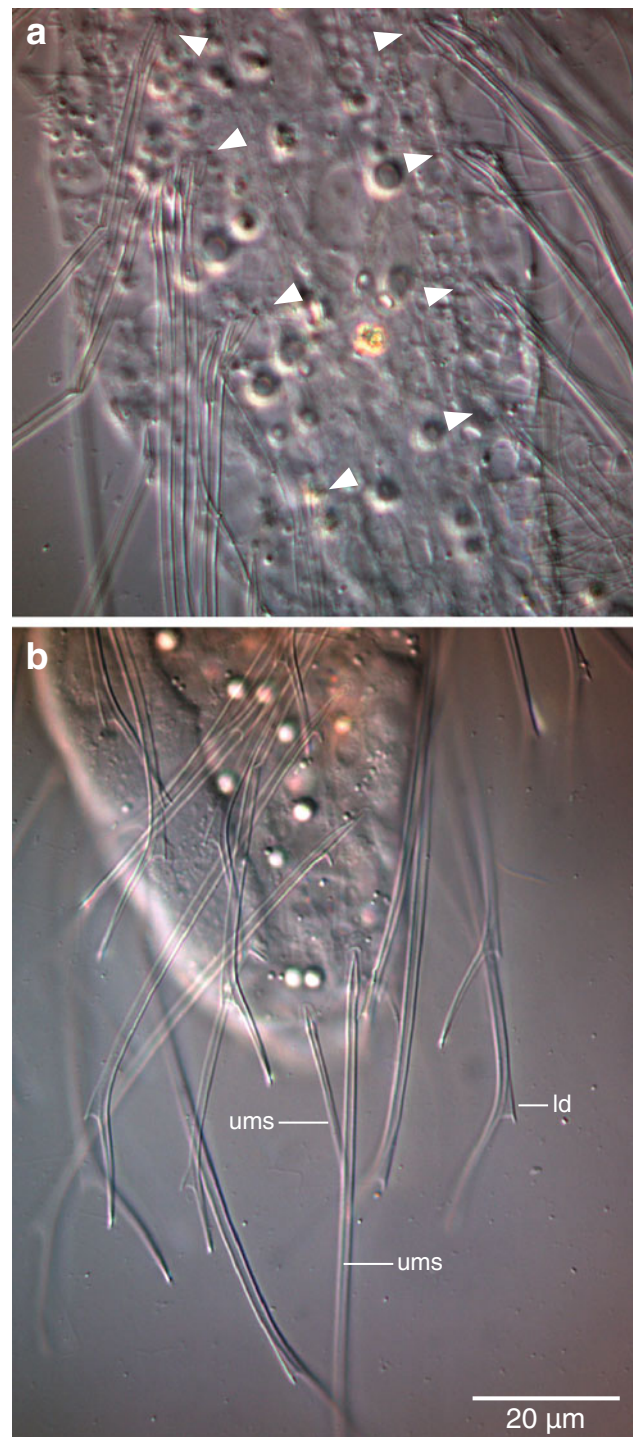

Fig. 2 Details of the motile spines of *Dasydytes goniathrix*. **a** *ventral view* of the spine insertions of groups ta–td (*white triangles*). **b** Rear trunk end, *dorsal view* (both images: differential interference contrast optic). *ld* lateral denticle of trunk spine, *ums* unpaired medial dorsal spines

and three spines in the cephalic and neck spine groups (*ca1-2*, *cb*, *cc1-3*), one to five spines in the trunk spine groups (*ta1-4*, *tb1-5*, *tc1-4*, *td1-3*, *te1-2*, *tf*) and a single pair
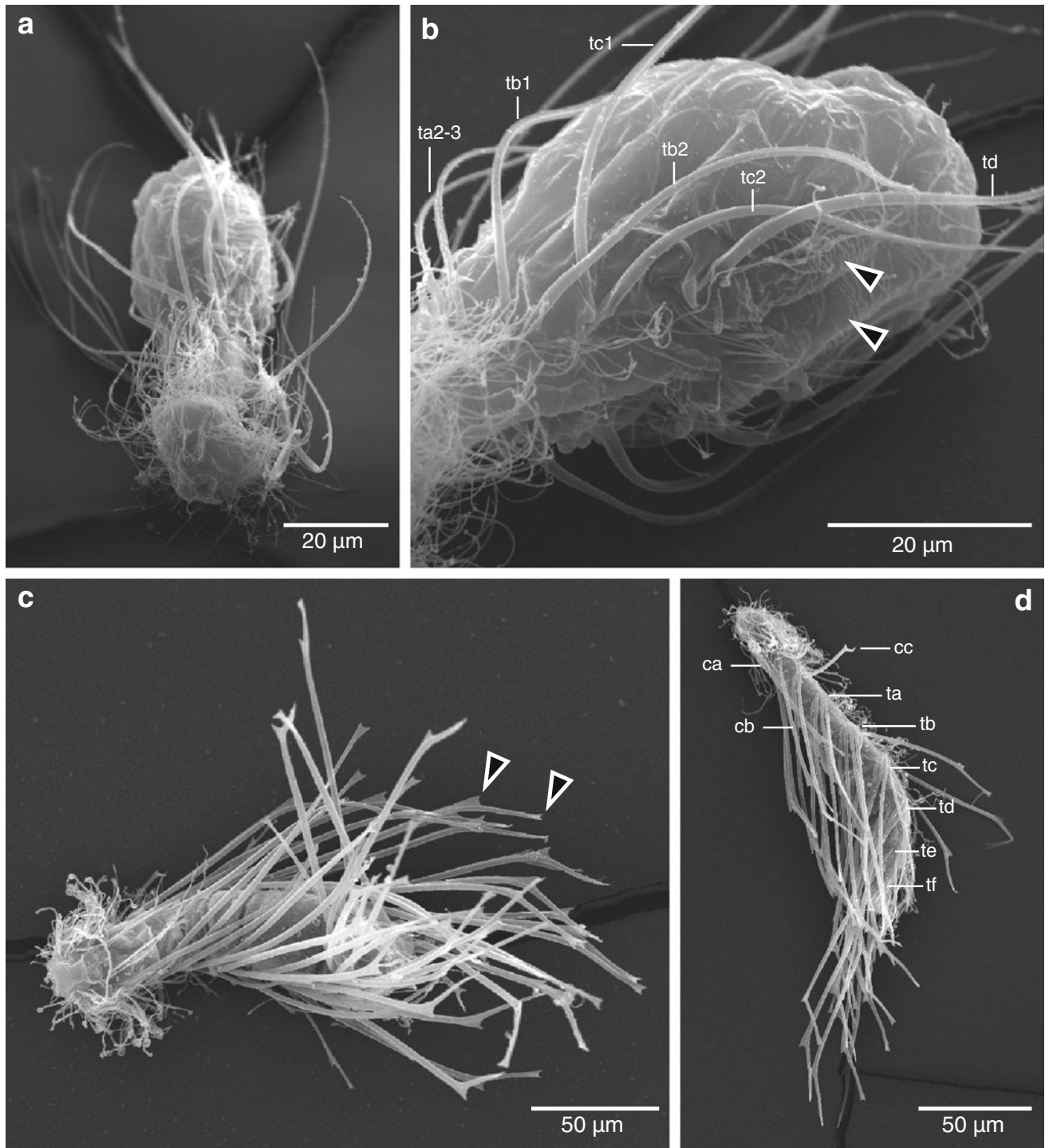
Fig. 3 Scanning electron microscopy of the investigated species. **a** *Haltidytes crassus*, whole animal from frontal. *Ventral side* to the *right*. **b** *H. crassus*, details of the trunk spine insertions, seen from ventrolateral. Note the massive ridges on the ventral side of the rear trunk produced by musculi laterales segmentum V (*triangles*). **c** *Dasydytes goniathrix*, whole animal seen from dorsal. Note the lateral denticle and the bifurcate apex of the spines (*triangles*).

d *D. goniathrix* seen from the *right side* (*ventral side* to the *right*). *ca* first group of head plus neck spines, *cb* second group of head plus neck spines, *cc* third group of head plus neck spines, *ta* first group of trunk spines, *ta2-3* second and third spines of group ta, *tb* second group of trunk spines, *tb1* first spine of group tb, *tb2* second spines of group tb, *tc* third group of trunk spines, *tc1* first spine of group tc, *tc2* second spine of group tc, *td–tf* fourth to sixth group of trunk spines

of rear trunk spines (*r*). Additional unpaired mid-dorsal spines were observed especially in the rear trunk region (Figs. 1c; 2b). Spines of each group are stiff and solid and possess a bifurcate apex and one lateral denticle at the beginning of the distal quarter of its length where each spine is kinked at an angel of ca. 35° (Figs. 2b; 3c, d). Spine length increases only slightly from anterior to posterior. However, there are few considerable shorter spines in the first cephalic and posterior trunk groups. Spine length per group increases from ventral to dorsal.

With a total body length of ca. 180 μm, *D. goniathrix* are rather big animals and steady swimmers, for which again the incomplete ciliary rings on the head section and ventral tufts of cilia are used (Figs. 1c; 3c). During change in direction, the head is tilted, the whole trunk bends ventrally, and the motile spines are slightly abducted. When disturbed, *D. goniathrix* performs a "defense position": The whole trunk is curled ventrally, and the motile spines are spread to a maximum. Stretching of the trunk and spine adduction occur relatively slow compared to, for example, *H. crassus*.

Musculature

The musculature of *H. crassus* and *D. goniathrix* consists of individual muscles in circular, helicoidal, longitudinal, oblique and dorsoventral orientations arranged as somatic (body wall) and visceral (gut) components. The somatic component consists of one pair of segmented or partitioned longitudinal muscles (musculi laterales), one pair of branched dorsodermal muscles (*R1*, *R2*) and several pairs of oblique muscles in the trunk. The visceral component includes helicoidal muscles, three pairs of longitudinal muscles (musculi ventrales, m. ventrolaterales, m. dorsales) and a pair of dorsoventral muscles in the posterior. Visceral circular muscles surrounding the pharynx could not be detected, but their existence is possible.

Haltidytes crassus

Somatic muscle components

In a ventrolateral position, *H. crassus* has two pairs of longitudinally arranged muscle strands. Based on a comparison of muscular characters of other species of the Dasydytidae (see discussion below), these two paired longitudinals are identified as the first and fifth segments of the segmented musculi laterales (*ml I* and *ml V*). The first pair (*ml I*) extends from the head to the insertions of the first group of trunk spines (∼U0 to U30). At approximately half muscle length, *ml 1* divides to produce a curved branch that inserts in the ventrolateral region of the third head lobe (Figs. 4c; 5a–d). There is a second pair of somatic longitudinal muscles (*ml V*) that span from the fourth pair of trunk spines to almost the caudal end of the animal (U60–U95). This considerably strong muscle pair can even be recognized externally on the specimens prepared for SEM (Fig. 3b). In a dorsolateral position, there is a further pair of somatic longitudinal muscles. Seen from the side, this dorsodermal muscle or *R1* (the old term 'Rückenhautmuskel' introduced by Zelinka 1889 was still in use, see, e.g., Hochberg and Litvaitis 2003, but has recently been replaced by Leasi and Todaro 2008) extends in an arch along the dorsal integument, starting laterally in the region between the first and second groups of trunk spines (U40) and ending close to caudal end (Figs. 4a, 5a, b, d, 6a). This strong muscle (diameter between 1.5 and 2.5 μm) produces a branch at two-thirds of its length (at U75). This muscular branch, the *R2*, runs ventrally along the integument. In contrast to most other Paucitubulatina species, the dorsodermal muscle of *H. crassus* is neither attached frontally nor caudally to the visceral musculi dorsales (see below).

Most conspicuous are the serially arranged paired oblique muscles. In *H. crassus*, there are four pairs of somatic musculi obliqua, *mo I–mo IV* (Figs. 4a–c; 5a–d; 6a, b). All four oblique muscles have slightly posterior origins on the dorsolateral body wall: *mo I* has a trifurcate origin, *mo II* has a bifurcate origin, and *mo III* and *mo IV* have singular origins. All four muscles extend anteriorly and insert on the ventral body wall, close to the insertions of the four trunk spine groups (between U30 and U60). The first oblique muscle pair (*mo I*), seen from a lateral orientation, has an angle of ca. 25–30° with the main body axis. This angle successively decreases toward the last oblique muscle pair (*mo IV*), that is almost parallel to the longitudinal musculus lateralis segmentum V. Both anterior muscle pairs are flat, sail-like structures. The two posterior oblique muscles (*mo III* and *mo IV*) are rather rod-shaped, solid muscle strands. In addition, a pattern of short bulges at the ventrolateral ends of musculi obliqua is observable. The number of these bulges coincides with the number of motile spines in the related spine group: *mo I* has three bulges, *mo II/III* have two bulges each, and *mo IV* has a single bulge only (Figs. 4b, c; 5a, c).

Visceral muscle components

The strongest fluorescence signal is produced by the phalloidin-labeled myoepithelium of the pharynx that occupies the whole head plus neck region (U0–U30). Due to the strength of the signal, we were not able to determine whether there are circular muscles lining the pharynx. There are two ventral pairs and one dorsal pair of longitudinal muscles extending to the whole length of gut tube (pharynx plus intestine): musculi ventrales, musculi ventrolaterales and musculi dorsales (Figs. 4c; 6a, b).
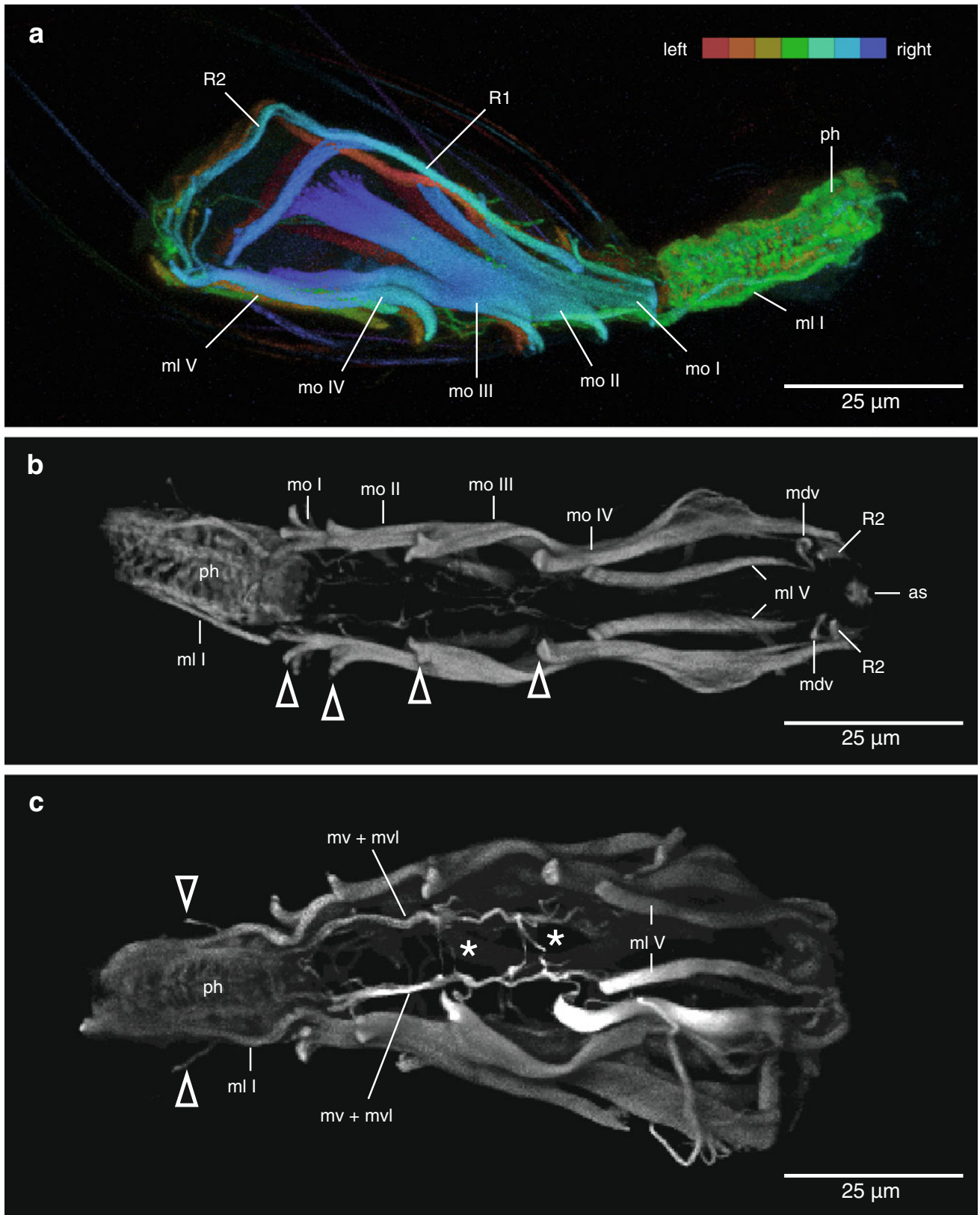
◀ **Fig. 4** *Haltidytes crassus*, fluorescence signals of the actin filaments, stained by TRITC–phalloidin. **a** Maximum projection of a complete image stack, color-coded by depth (see color code *top right*). Specimen seen from its *right side*. **b, c** Two further specimens, seen from ventral. Maximum projections of a subset of the image stack (ventral half of images). Note the proximal insertions of the oblique muscles in b (*triangles*) and the crossings of the helicoidally arranged muscle fibers enwrapping the anterior portion of the midgut (*asterisks*) as well as the lateral branching of musculi laterales segmentum I in c (*triangles*). *as* anal sphincter, *mdv* dorsoventral muscles, *ml I* musculi laterales segmentum I, *ml V* musculi laterales segmentum V, *mo I–mo IV* musculi obliqua I–IV, *mv* musculi ventrales, *mvl* musculi ventrolaterales, *ph* myoepithelial pharynx, *R1* dorsodermal muscle branch 1, *R2* dorsodermal muscle branch 2

Frontally, each muscle is attached to the integument close to the almost terminal mouth opening, while musculi dorsales and musculi ventrolaterales are caudally coupled with a small muscle ring, the sphincter. Close to its posterior end, musculi ventrales appear to bend sharply, first dorsal and then ventral. It is possible that there is a minute dorsoventrally oriented muscle pair, connecting musculi ventrales and musculi ventrolaterales next to their posterior ends (Fig. 4b). However, due to the small body size of the studied animals and ambiguous signals in this region of the body, the exact course of muscles and existence of the posterior dorsoventral muscle has to be verified elsewhere. Outside the three pairs of visceral longitudinal muscles, there is a pair of spirally arranged muscles forming the characteristic muscle helix of the Gastrotricha (Fig. 4c). In *H. crassus*, these muscles are present from the pharyngeointestinal junction to approximately half of the intestine (U30–U75) and consist of 5–6 crossings (dorsal plus ventral). We were not able to find any trace of these muscles in the region of the pharynx. However, as stated earlier, the fluorescence signals of the pharynx are very strong and may shield finer patterns.

In all muscle components (somatic and visceral), apart from the R1/R2, the fluorescent signals were almost uniformly bright over the whole length of each muscle. There was hence no obvious arrangement pattern of the contractile elements. However, both branches of the dorsodermal muscle indeed show a spiraled arrangement of the stained actin filaments in different specimens corresponding to obliquely striated muscles R1 and R2.

Dasydytes (Dasydytes) goniathrix

Somatic muscle components

Ventrolaterally, there is a paired column of aligned longitudinal muscle sections, the segmented musculi laterales (Figs. 7a, b; 8a–d; 9a, b). In total, there are ten muscle segments per side, musculus lateralis segmentum I–X. While segments IV–X are parallel to the main body axis,

the anterior three segments more or less follow the contours of the head region (Figs. 7a; 9b). Apart from the first segment that is attached to the body wall close to the mouth opening, each segment of musculi laterales is frontally associated with the bases of motile spines and caudally with an oblique muscle and the spines of the following group. In this area, many of the segments of musculi laterales (*ml IV–ml VIII*) have a short, dorsolaterally projecting extension (Figs. 7a; 8a, c; 9a, b). However, we were not able to clarify whether the segments of musculi laterales and the oblique muscles are directly attached to the spine bases or whether they just terminate in the body wall close to them. The posterior-most segment of musculi laterales (*ml X*) caudally terminates close to the body wall. While the anterior- and posterior-most segments are the shortest, the lengths of the segments do not vary that much and the "gaps" between adjacent segments are more or less equally distributed along the trunk (Figs. 7a; 8a, d).

In a dorsolateral position, there is another somatic longitudinal muscle pair, the dorsodermal muscle (*R1*). In *D. goniathrix*, it branches off from the visceral musculi dorsales at approximately U30, runs in a wide arch along the dorsal integument and reunites with musculi dorsales at U90 (Figs. 7b; 8a; 9a). At approximately U35, a short muscle strand connects the *R1* with musculi dorsales. This muscle connection most likely represents a strongly reduced *R2* branch of the dorsodermal muscle (Figs. 7b; 8a; 9a).

Dasydytes (Dasydytes) goniathrix also possesses pairs of serially arranged oblique muscles. There are six pairs of these simple, rod-shaped muscles (musculi obliqua III–VIII), which are, with their proximal ends, positioned between two adjacent segments of musculi laterales (hence in the region of trunk spine insertions) and extend dorsocaudally along the lateral integument (Figs. 7a, b; 8a). In the head and neck region, there are two further muscular fibers associated with the three anterior segments of musculi laterales (*ml I–ml III*). The first fiber stretches from the posterior tip of ml I to the posterior tip of *ml II* (in lateral view, these three muscles form a triangular structure, Figs. 8a–c; 9a), and the second fiber is more or less parallel with *ml III*. We interpret these muscles as the anterior two pairs of oblique muscles (*mo I–II*). Hence, *D. goniathrix* possesses eight pairs of musculi obliqua in total (*mo I–mo VIII*, Fig. 9a, b). Lengths of musculi obliqua slightly increase from 10 to 15 μm (*mo I–mo IV*) up to 20 μm (*mo V*) and then decrease again (*mo VI–mo VIII*).

Visceral muscle components

As in the previous species, the strong myoepithelial pharynx (U0–U25) yields the strongest fluorescence signal, and we were not able to verify the existence of a circular
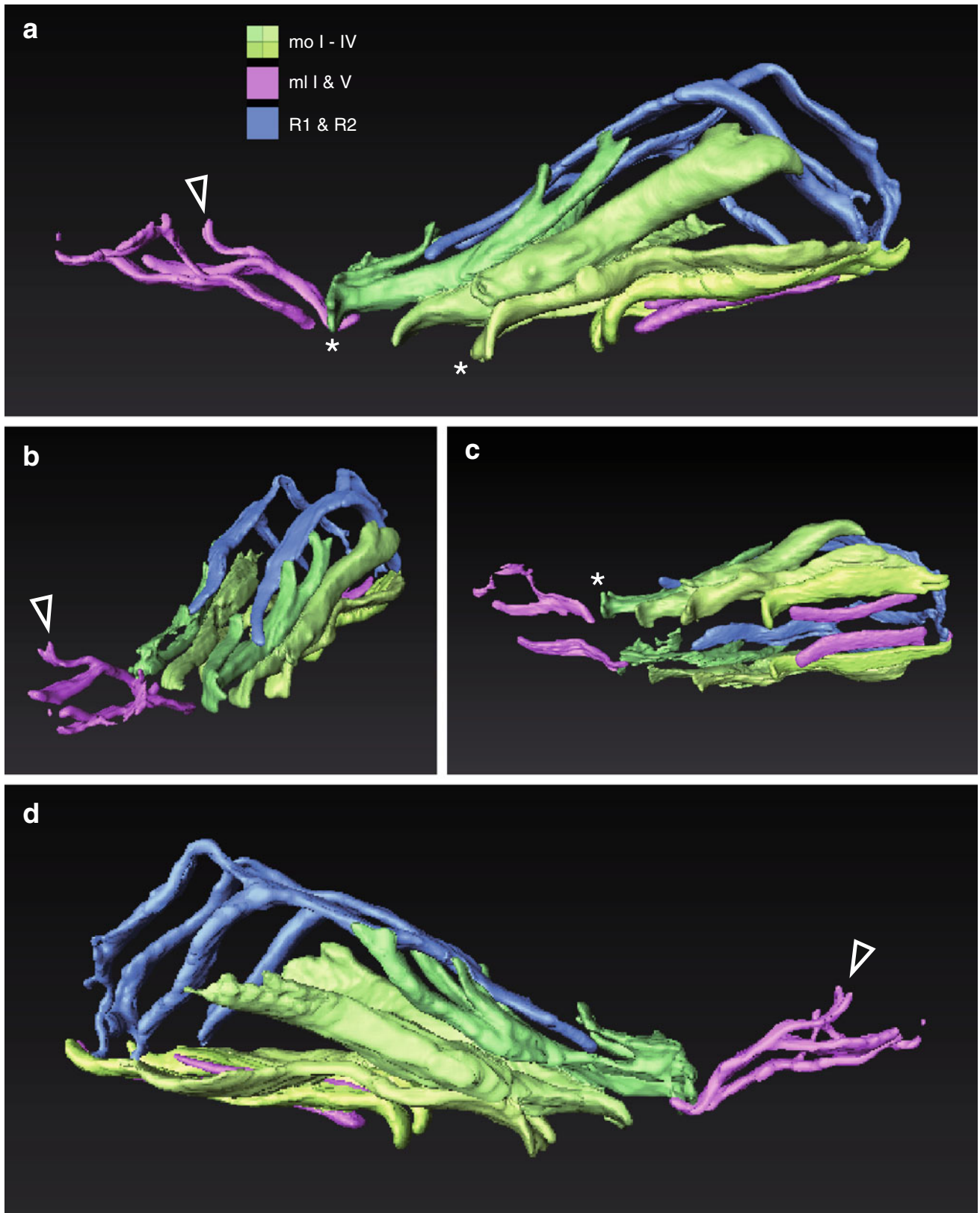
muscle sheath surrounding the foregut. There are also three
pairs of longitudinal visceral muscles in *D. goniathrix*
spanning the whole gut tube: musculi dorsales, musculi
ventrales and musculi ventrolaterales (Figs. 7a, b; 9a, b).
Attachment patterns are the same as in *H. crassus* (frontally
attached to the integument close to the mouth opening,
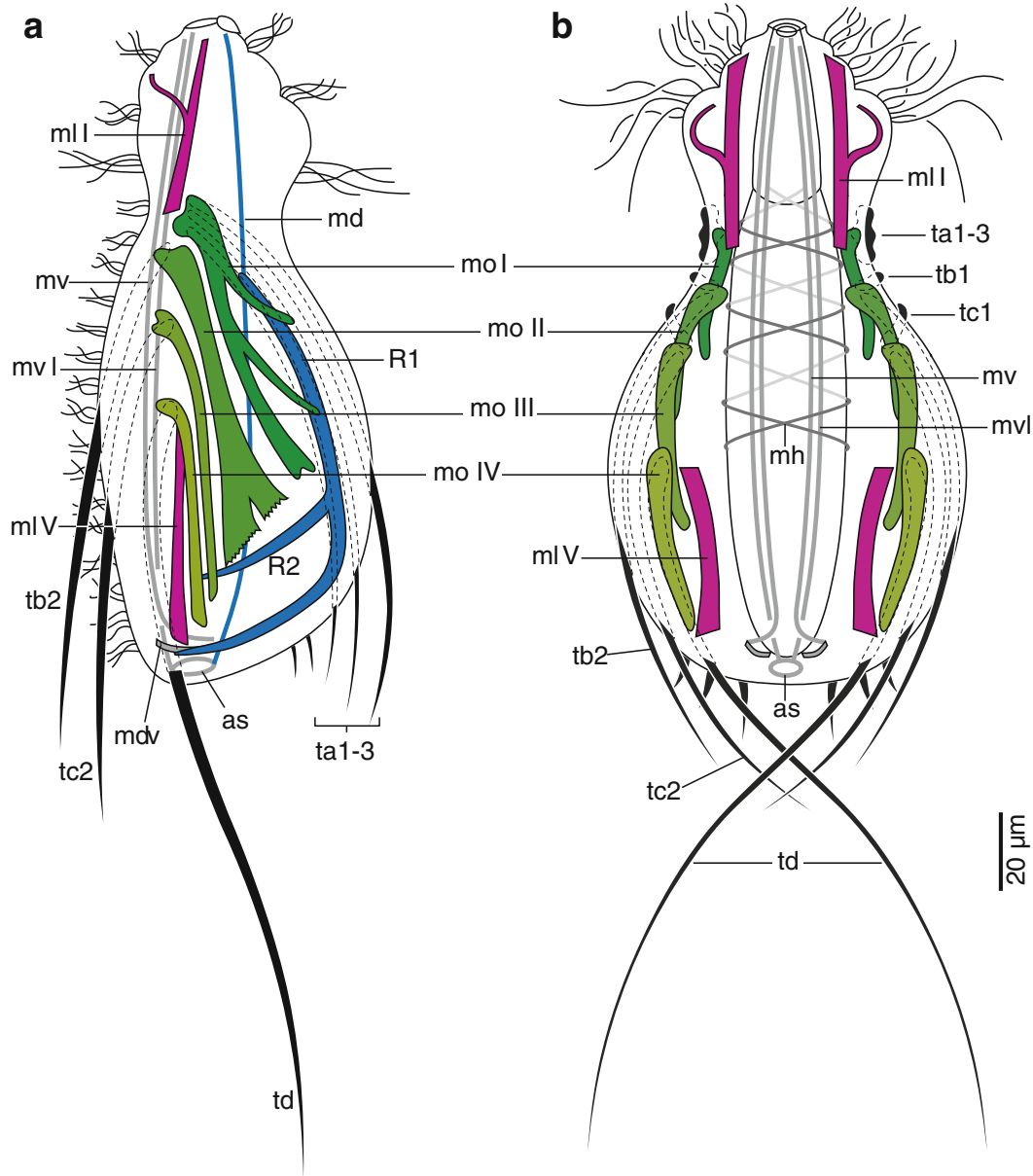caudally attached to the sphincter (*md*, *mvl*), and there is as

Fig. 6 *Haltidytes crassus*, schematic illustration of the whole muscular system. **a** *Lateral view* (*left side*). **b** *Ventral view*. Note that for simplicity, not all spines are fully drawn. *as* anal sphincter, *md* musculi dorsales, *mdv* dorsoventral muscles, *ml I* musculi laterales segmentum I, *ml V* musculi laterales segmentum V, *mo I–mo IV* musculi obliqua *I–IV*, *mv* musculi ventrales, *mvl* musculi ventrolaterales, *R1* dorsodermal muscle branch 1, *R2* dorsodermal muscle branch 2, *ta1-3* first, second and third spines of group ta, *tb1* first spine of group tb, *tb2* second spine of group tb, *tc1* first spine of group tc, *tc2* second spine of group tc, *td* single spine pair of group td

well the sharp dorsal–ventral bend of musculi ventrales at their posterior end (Fig. 7a, b)). The presence of a short caudal dorsoventral muscle pair is also likely (see Fig. 7a). External to the aforementioned muscle components, there is also the muscular double helix (Fig. 7a, b). However, it seems to be restricted to the first third of the midgut (U25–U50), and we were able to count five crossings in total (dorsal plus ventral).

In all muscle components (somatic and visceral), the fluorescent signals were more or less uniformly bright over the whole length of each muscle. There was no striation pattern of the contractile elements.

Phylogenetic analysis

Fifty-one out of the 80 characters were parsimony-informative (7 constant and 22 parsimony-uninformative variable characters). The heuristic search found 37 equally parsimonious trees with a length of 142 steps (CI: 0.831, RI: 0.843), and half of these were found on the same tree island. As we have put the focus on the Dasydytidae, we here give a description on the implications for possible internal relationships of this group exclusively, whereas other taxa that are not part of that group serve as an "emended outgroup". The summarized topology of the 50% majority rule consensus tree is as follows (Fig. 10); to ease the description, we use the genus names only. According to the results of our analysis, Dasydytidae form a well-supported monophyletic group (bootstrap value of 100% plus nine unambiguous character transformations). There are three major lineages within Dasydytidae: (a) a clade comprising *Stylochaeta*, *Anacanthoderma* and *Chitonodytes*, (b) a clade comprising *Dasydytes*, *Setopus* and *Ornamentula*, and (c) the taxon *Haltidytes*. These three lineages are moderately supported by bootstrap values (56, 65 and 64% for clades a, b and c) and each characterized by different putative autapomorphies (2, 2 and 7 unambiguous character transformations for clades a, b, and c, see Fig. 10). Apart from the sister group relationship of *D. goniathrix* and *S. tongiorgii* (bootstrap support of 54% plus five unambiguous character transformations), other groupings within clades a and b do not get strong support (bootstrap values below 50%, few unambiguous character transformations). The situation is almost similar regarding the sister group relationship between clades b and c (i.e., *Haltidytes*) (Fig. 10).

The stem species and character evolution of Dasydytidae

To get an idea of possible evolutionary progress among Dasydytidae, one can follow consecutive autapomorphies from the base of the hypothesized phylogenetic tree toward its terminals (Fig. 10). Initial point for evolution is the last common ancestor (the stem species). Based on the present data set, the consensus tree topology and the criterion of maximum parsimony as implemented in the Mesquite module, we have reconstructed the putative character vector (plesiomorphic and apomorphic character states) of the stem species of Dasydytidae (see supplementary file 1). Just a few characters had ambiguous results for the reconstructed character state (shown as multiple entry or '?' in the matrix).

The stem species was a microscopic, planktonic animal (autapomorphy of Dasydytidae, AA) with a tenpin-shaped body (AA) and a more or less rotund trunk in cross section (AA). There were four anterolateral tufts plus two posterolateral bands of cilia on the head region (AA) as well as cilia on the ventral side of the trunk, either as two longitudinal rows or as longitudinally arranged paired patches of cilia. Ciliary swimming movements (AA) were supported by the action of four paired groups of motile trunk spines (AA) that possessed one or two laterally directed denticles per spine. Rear spines were not present. The four groups of trunk spines were associated with five pairs of segmented musculi laterales (AA) and apparently with four pairs of musculi obliqua (AA). Apart from this specialized somatic musculature, the stem species of Dasydytidae had further visceral muscle components: longitudinal paired musculi dorsales (one pair of somatic dorsodermal muscles, R1, branches off from m. dorsales), m. ventrales and m. ventrolaterales, circular muscles enwrapping the myoepithelial pharynx, and a pair of posterior dorsoventral muscles. The muscular double helix that lies outside the visceral longitudinal muscles could be restricted to the mid-intestine region (AA). Even though ventral scales in the trunk region could explicitly be reconstructed, it still remains unclear whether the common ancestor of Dasydytidae had scales elsewhere on his body surface.

Discussion

Musculature in Dasydytidae

The results of the present study support the findings of an earlier investigation of the muscular system of two dasydytid gastrotrich species, *Stylochaeta scirtetica* and *Setopus tongiorgii* (Kieneke et al. 2008a). Like these, the two investigated species also possess three pairs of visceral longitudinal muscles, a single pair of posterior dorsoventral muscles, a visceral helicoidal musculature, somatic dorsodermal muscles and one pair of partitioned somatic longitudinal muscles plus serially arranged oblique muscles that are associated with the motile spines. The two latter characters are unique features among Gastrotricha and were reconstructed as putative autapomorphies for Dasydytidae. However, a peculiarity in these characters was
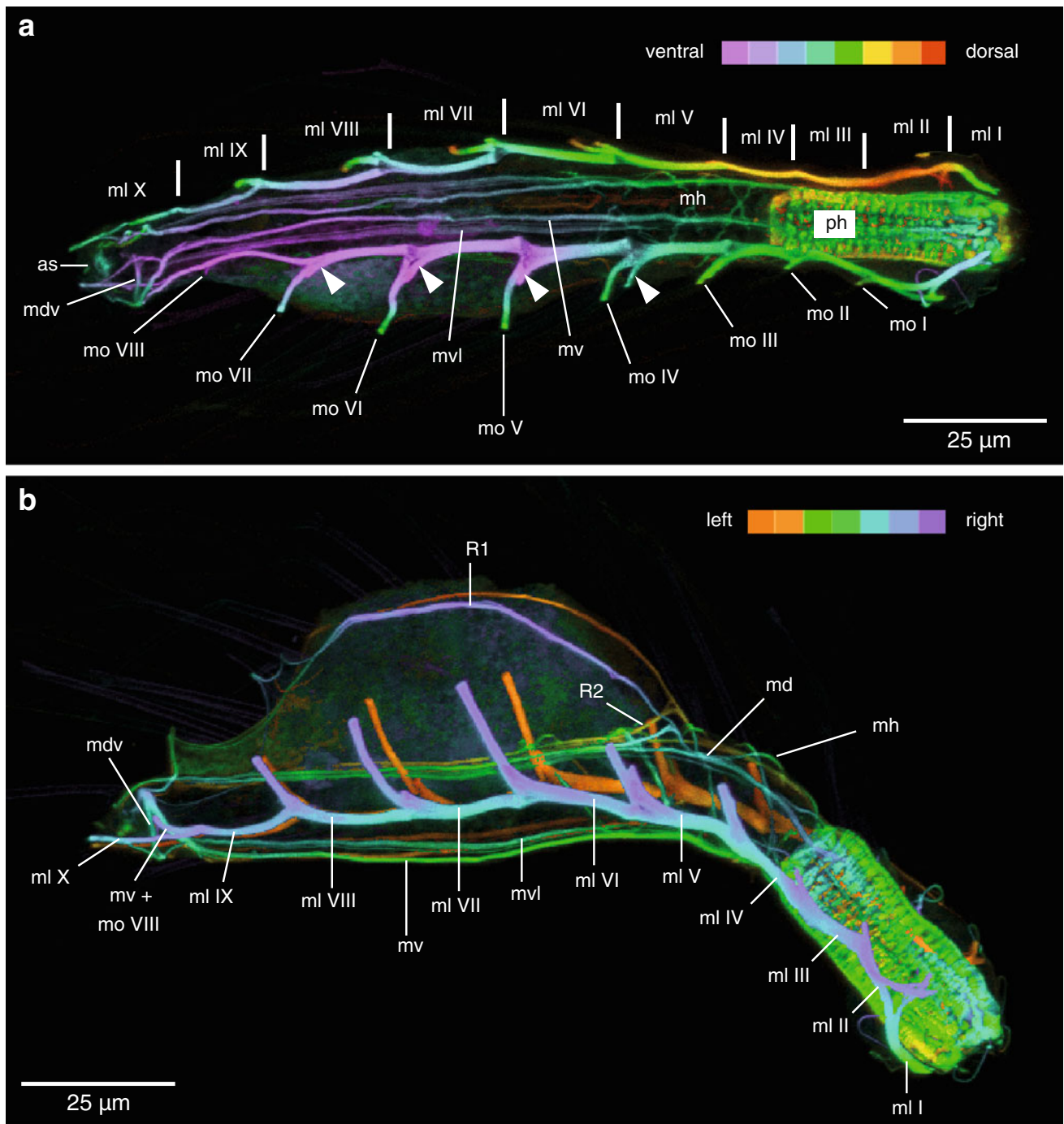
Fig. 7 *Dasydytes goniathrix*, fluorescence signals of the actin filaments of two different specimens, stained by TRITC–phalloidin. **a** Maximum projection of a complete image stack, color-coded by depth (see *color* code *top right*). Specimen seen from its *ventral side*. Note the distal branching of several segments of musculi laterales (*white triangles*). **b** Maximum projection of a complete image stack, color-coded by depth (see *color* code *top right*). Specimen seen from its *right side*. *as* anal sphincter, *md* musculi dorsales, *mdv* dorsoventral muscles, *mh* musculi helicoidales, *ml I–ml X* musculi laterales segmentum I–X, *mo I–mo VIII* musculi obliqua I to VIII, *mv* musculi ventrales, *mvl* musculi ventrolaterales, *ph* myoepithelial pharynx, *R1* dorsodermal muscle branch 1, *R2* dorsodermal muscle branch 2

observed in *H. crassus*: In this species, the segments of the musculi laterales spanning between the proximal ends of the motile spines (i.e., ml II–ml IV) are absent. Since all other species studied so far possess longitudinal muscles that connect the spine bases, we interpret their absence in *H. crassus* as a secondary loss. Also, the presence of incomplete somatic circular muscles associated with the motile spines in *D. goniathrix* as suggested by Remane

(1936) cannot be supported. Somatic circular muscles (complete or incomplete) or dorsoventral muscles commonly occur in species of putative basal groups of Paucitubulatina such as Xenotrichulidae and *Musellifer* (Hochberg and Litvaitis 2001b, 2003, Leasi and Todaro 2008, 2009) and many species of the marine Macrodasyida (e.g., Hochberg and Litvaitis 2001c, Leasi et al. 2006) and *Neodasys* (Hochberg 2005). Although the homology criterion of position could argue for a homology of somatic circulars and oblique muscles, we do not consider them homologs. Leasi and Todaro (2008) have reconstructed an evolutionary sequence within Paucitubulatina from almost complete somatic circular muscles in *Musellifer* via dorsoventral muscles in members of the Xenotrichulidae toward a complete loss of this muscle component in Chaetonotidae (the putative basal chaetonotid taxon *Polymerurus*, however, still possess visceral dorsoventral muscles). Since species of Dasydytidae are probably related to Chaetonotidae or at least a part of that taxon (see Hochberg and Litvaitis 2000, Kieneke et al. 2008b, Kånneby et al. 2012), we think that the absence of circular (dorsoventral) muscles in a somatic position is also a plesiomorphic feature for Dasydytidae. Hence, oblique muscles are probably a new formation.

The presence of the other longitudinal muscles of Paucitubulatina (see, e.g., Hochberg and Litvaitis 2003, Leasi et al. 2006) was confirmed for the two species studied here: musculi ventrales, m. ventro-laterales, m. dorsales and both branches of the dorsodermal muscle, R1 and R2. However, regarding the latter muscle component, both species show peculiarities: In *H. crassus*, both branches of the dorsodermal muscle are strongly developed but are not connected to the musculi dorsales, and in *D. (Dasydytes) goniathrix*, the R2 branch is only a very short connection from R1 to the musculi dorsales. In the two other dasydytid species studied so far, *S. cirtetica* and *S. tongiorgii*, there are also deviations of the "normal" occurrence: *S. scirtetica* completely lacks the R2 branch, and in *S. tongiorgii*, the R2 is connected neither to R1 nor to musculi dorsales (Kieneke et al. 2008a). A fifth pair of longitudinal muscles are as yet only known from Xenotrichulinae (the visceral musculi ventromediales, see Hochberg and Litvaitis 2003) or from *Draculiciteria tesselata* (Renaud-Mornant, 1968), respectively (the musculi paralaterales, see Hochberg and Litvaitis 2001b). Such muscles were also absent in the studied dasydytids.

If the interpretation of the data presented here and in the study of Kieneke et al. (2008a) is correct and the helicoidal muscles are absent from the pharynx in the studied species of the Dasydytidae, this could mean a unique (apomorphic) character for this group (see "Results"). A possible absence or presence of visceral circular muscles in the pharyngeal region of certain species of the Dasydytidae needs to be verified with ultrastructural investigations since the resolving power of the confocal microscope is limited by the strong fluorescence signal of the myoepithelial pharynx.

Functional aspects

While most gastrotrichs have a substratum-bound life (i.e., interstitial, epibenthic or periphytic), there exist some freshwater taxa that live in the water column of small stagnant inland waters. Among these groups that are commonly assigned to be semiplanktonic gastrotrichs (although the attributes 'hyperbenthic' and 'periphytic' are also applicable, see, e.g., Balsamo and Todaro 2002), the taxon Dasydytidae is additionally characterized by the presence of long and motile spines that support locomotion and defense. There is no doubt that a system of spine-associated oblique and partitioned lateral muscles is engaged in bringing about spine action. Despite several similarities between the body muscle patterns of the four species of Dasydytidae hitherto investigated, there are differences worth noting. These differences, such as the shape of oblique muscles or the absence of certain elements, are most likely related to differences in their functionality. Based on the morphological data of *S. scirtetica* and *S. tongiorgii*, an antagonistic role of the oblique and segmented lateral muscles in the spine abduction–adduction cycle was hypothesized (Kieneke et al. 2008a). However, with the new structural data and observations of two further species of the Dasydytidae obtained in this study, this simplified view of the abduction–adduction cycle has to be questioned. *S. scirtetica* is the only species where the observed mode of spine action (a quick spread and a likewise fast recovery of spines) and the musculature-spine morphology is in concordance with an abduction–adduction cycle mediated by muscular antagonists according to Kieneke et al. (2008a). Similarly, for the planktonic rotifers *Hexarthra* cf. *mira*, *Polyarthra* cf. *vulgaris* and *Polyarthra major* Burckhardt, 1900, an antagonistically working system of abductors and adductors was hypothesized for enabling the movements of their limb-like (in *Hexarthra*) or paddle-like (in *Polyarthra*) appendages (Hochberg and Gurbuz 2008). However, in *D. (Dasydytes) goniathrix*, the recovery movements seem to be a passive process that does not involve direct muscle action. It is questionable whether the lateral muscle segments are stretched at all when spines are spread so that they even may not be able to function as adductors. Slight and maximum abduction of motile spines in *D. goniathrix* and in *S. tongiorgii* (due to striking morphological similarity in both species, we suspect a comparable mechanism) in contrast must be an active muscular process. Since slight abduction of spines of *D. goniathrix* was observed to occur
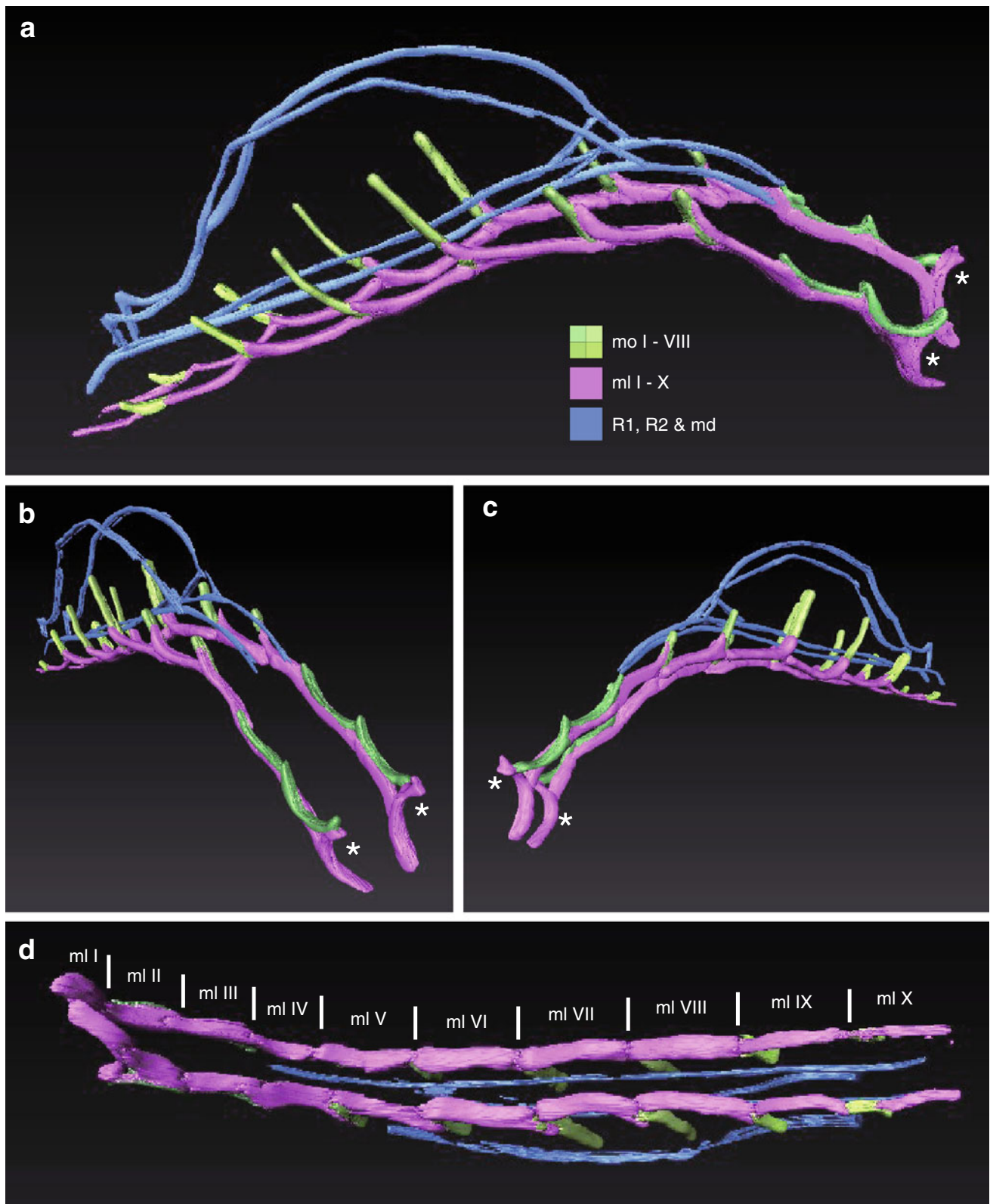
Fig. 8 *Dasydytes goniathrix*, three-dimensionally reconstructed somatic musculature. **a–d** Surface model seen at different viewing angles. *Color* code for the different muscular components according to the legend in (**a**). Note the special course of musculi laterales segmentum I and musculi obliqua I that form a triangular structure (*asterisks*). *md* musculi dorsales, *ml I–ml X* musculi laterales segmentum I to X, *mo I–mo VIII* musculi obliqua I to VIII, *R1* dorsodermal muscle branch 1, *R2* dorsodermal muscle branch 2
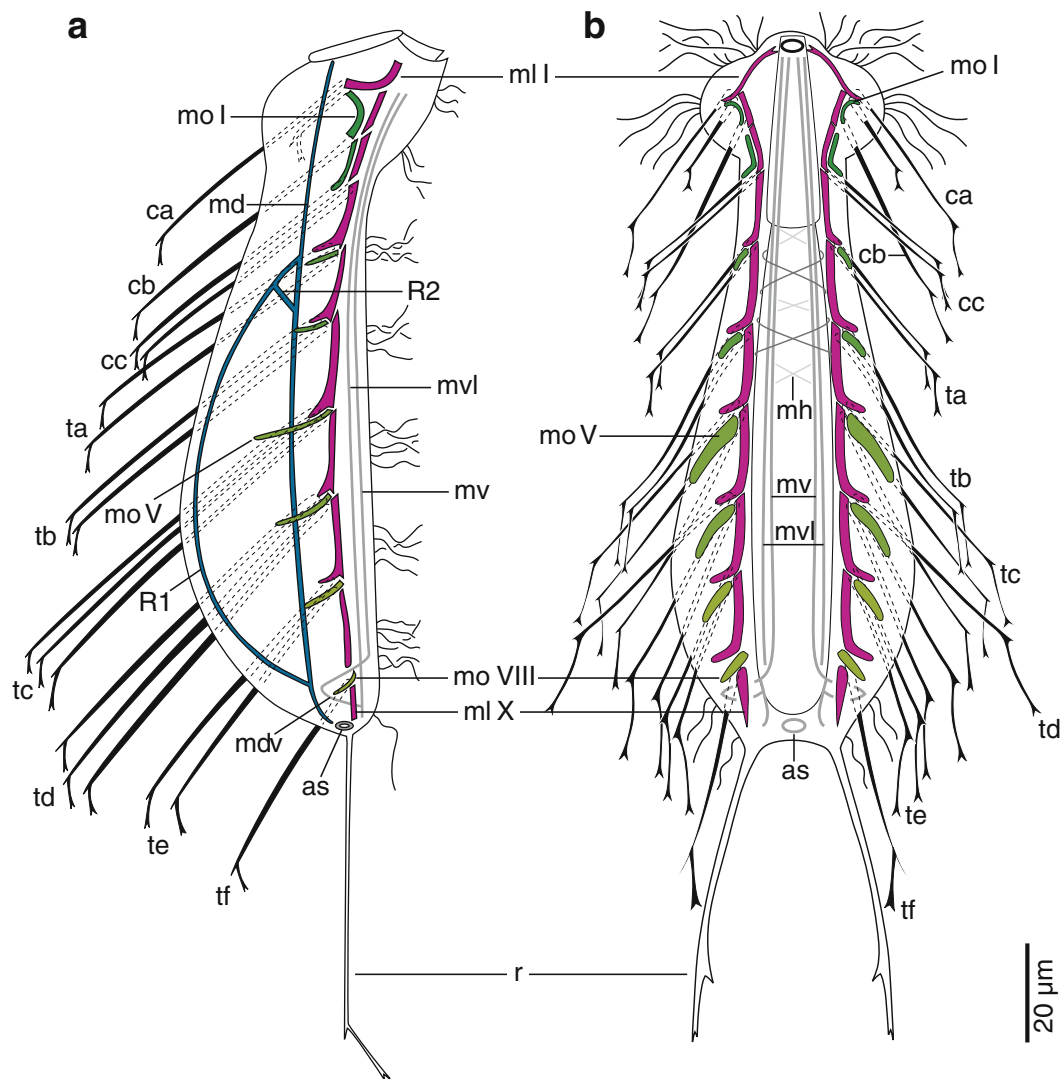
Fig. 9 *Dasydytes goniathrix*, schematic illustration of the whole muscular system. **a** *Lateral view* (*right side*). **b** *Ventral view*. Note that for simplicity, not all spines are drawn for each group. *as* anal sphincter, *ca* first group of head plus neck spines, *cb* second group of head plus neck spines, *cc* third group of head plus neck spines, *md* musculi dorsales, *mdv* dorsoventral muscles, *mh* musculi helicoidales,

ml I musculi laterales segmentum I, *ml X* musculi laterales segmentum X, *mo I* musculi obliqua I, *mo V* musculi obliqua V, *mo VIII* musculi obliqua VIII, *mv* musculi ventrales, *mvl* musculi ventrolaterales, *r* rear spines, *R1* dorsodermal muscle branch 1, *R2* dorsodermal muscle branch 2, *ta–tf* first to sixth groups of trunk spines

simultaneously with trunk bending during changes in the locomotion direction, it might be possible that contractions of musculi laterales cause (1) the caudal bending of the animal and (2) the slight spreading of the spines into a frontal direction. In order to abduct the spines to a maximum (e.g., for achieving the defense position), musculi obliqua are contracted, too, which may cause a more laterally directed movement of the spines. This would imply a synergistically functioning somatic musculature in *D. goniathrix* and *S. tongiorgii* rather than antagonistic muscles. Spine recovery to their resting position could be brought about passively by flexibility of the whole body and cuticle when both musculi obliqua and m. laterales do relax.

In *H. crassus*, three segments of the musculi laterales are absent (segments II–IV), and hence, important components of an antagonistically working spine-related musculature are missing. Species of *Haltidytes* are the only gastrotrichs that are able to perform true "jumps" through the water column. The very fast and strong spine abduction in order to do these saltatory movements is doubtlessly brought about by the strong oblique muscles. Furthermore, we think that the posterior spines of group *td* are moved through synergistic contraction of both the oblique muscles (*mo IV*) and the last segment of the lateral longitudinal muscles (*ml V*). Both muscle pairs insert at the spine bases and the caudal integument and have an almost parallel course.

Hence, it is impossible that contraction of the one component causes the relaxation of the other, a necessary property of antagonistic muscles. Kisielewski (1991) states that the single forth spine pair (td) in *Haltidytes squamosus* is the saltatory one. This assumption is possibly supported by our finding that this spine pair is obviously supplied with two pairs of abductor muscles. The only muscles that, in principle, could represent antagonistic adductors are the first segments of musculi laterales. But this could only explain adduction of spines of the anterior group ta. Therefore, we suggest that the function of this muscle is for head movement, which appears to be the case for *S. scirtetica* and *S. tongiorgii* (Kieneke et al. 2008a). Recovery of raised spines in *H. crassus* could be achieved by an increased tissue pressure that is gained by contractions of, for example, both branches of the dorsodermal muscle that are as yet hypothesized to play a role in stabilizing and releasing mature eggs in species of Paucitubulina (Hochberg and Litvaitis 2003). We suspect strong cuticle deformations in the area next to the spine insertions when musculi obliqua of *H. crassus* are contracted and spines are raised. A muscular-mediated increase in tissue pressure could cause the cuticle to reach its initial position again, and by this, spines would be adducted. Such muscle-mediated but passive mechanisms accompanied by pressure changes in tissue/body fluid are also suggested for the movements of the long anterolateral setae of the planktonic rotifer *Filinia novaezealandiae* Shiel and Sanoamuang, 1993 (Hochberg and Gurbuz 2007).

In order to fully understand the sequence and control of spine action in the different species of Dasydytidae studied so far, we are in need of thorough life examinations carried out by video analysis as it was done for the mentioned planktonic rotifers. However, due to the velocity of movements in these microscopic animals, it was not possible to capture every "step" of the abduction–adduction cycle (Hochberg and Gurbuz 2008). Maybe high-speed video analysis techniques could provide advantages here. At the morphological level, it is still important to study the actual mechanic coupling of muscles and motile spines, if there are such couplings at all. These are mandatory if the muscle components would be effector organs, for example abductors or adductors.

Systematic and evolutionary aspects

Until now, there were only two systematic attempts focusing on (Kisielewski 1991) or covering all of the supraspecific taxa of Dasydytidae (Kieneke et al. 2008b). The gastrotrich phylogenetic paper of Hochberg and Litvaitis (2000) does not contain all dasydytid taxa; *D. (Prodasydytes)*, *Ornamentula* and *Setopus* were not considered in their analysis. The argumentation of the phylogenetic

hypothesis of Kisielewski (1991) is not in accordance with a strict phylogenetic approach. Some of the character sets indicated for a certain branch are plesiomorphic and cannot be used to form or define a monophyletic group, for example a clade comprising *Chitonodytes*, and both subtaxa of *Dasydytes* shall be characterized by the absence of ciliated protuberances at the caudal body end (Kisielewski 1991). However, such protuberances are absent in all taxa of Dasydytidae except for *Stylochaeta*, and hence, the absence of these 'styli' is a plesiomorphic condition. The phylogenetic study of Kieneke et al. (2008b) focused on the basal relationships of the Gastrotricha. Apart from some putative autapomorphies for the Dasydytidae, no character states for possible internal relationships were discussed or mentioned. According to the analysis results of that study, another planktonic taxon, the Neogosseidae, was placed as an ingroup taxon of Dasydytidae, a rather unlikely outcome. Furthermore, there is almost no statistic node support for internal branches. None of the DNA sequence-based phylogenetic inferences published to date include a single sequence of a member of Dasydytidae apart from the most recent one (Kånneby et al. 2012). In that study, sequences of two species of *Stylochaeta* form a monophylum within paraphyletic Chaetonotidae, with close relationships to *Polymerurus*.

Evolutionary patterns within the Dasydytidae were therefore exclusively analyzed using the phylogenetic hypothesis obtained by the present maximum parsimony analysis. With respect to the included outgroup species from various taxa of the Paucitubulatina, Dasydytidae form a well-supported monophyletic group. We have found three monophyletic lineages within Dasydytidae that were supported by moderate bootstrap values and different putative autapomorphies. A surprising result is the inclusion of *Anacanthoderma* within lineage a, that additionally comprises *Stylochaeta* and *Chitonodytes*. Kisielewski (1991) provisionally kept *Anacanthoderma* as a taxon affiliated to Dasydytidae but discusses a systematic position not strictly related to this group. In their recent taxonomic checklist of valid freshwater Gastrotricha, Balsamo et al. (2009) class *Anacanthoderma* among the Dasydytidae (see also Todaro 2012). The results of our analysis yield support for such a classification. However, it has to be stressed that *Anacanthoderma* is a poorly known taxon, that is, there might exist characters that have not been discovered yet, and which could argue for a systematic position elsewhere. Hence, it would be very interesting to investigate whether muscular patterns will support the hypothesized relationship of *Anacanthoderma* and Dasydytidae. The polyphyly of *Setopus* as revealed by the present analysis is possibly biased by heterogeneous data availability. Within lineage b, *S. tongiorgii* and *D. goniathrix* form sister groups and are the only terminals of that clade with available muscular data.
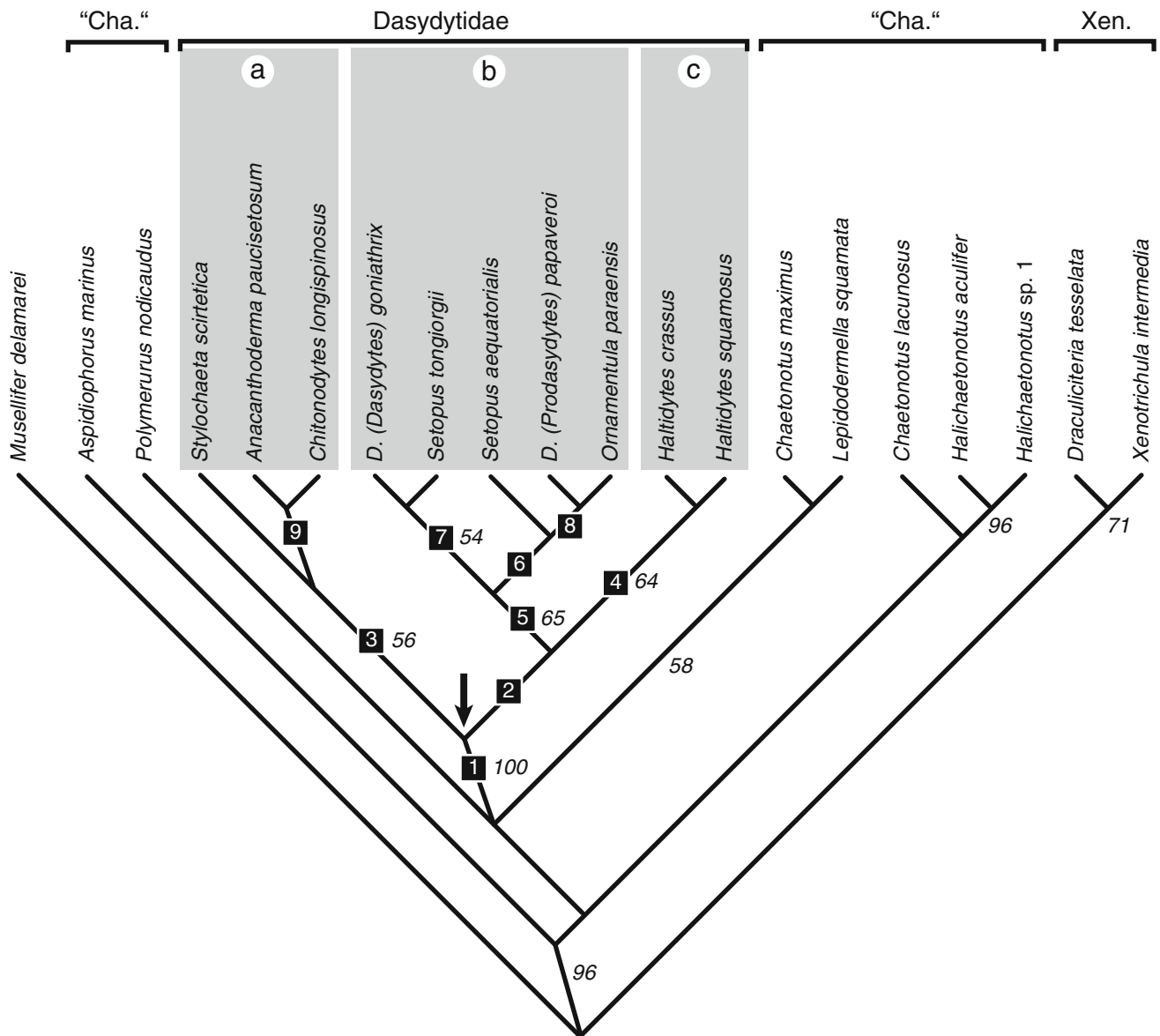
Fig. 10 Majority rule consensus tree (50%) calculated from 37 equally parsimonious trees (tree length: 142 steps, CI: 0.831, RI: 0.843) of the maximum parsimony analysis showing possible internal phylogenetic relationships of the Dasydytidae. Numbers in *italics* indicate the percentage recovery values of branches among 2,000 bootstrap replicates (only values higher than 50% are shown). Three moderately supported lineages (*a*, *b*, *c*) could be resolved within Dasydytidae. Numbered *black squares* indicate possible autapomorphies (unambiguous character transformations) for the Dasydytidae and internal groupings. In few cases, ambiguous transformations are also quoted (in *brackets*). *1* semipelagic to planktonic lifestyle, ciliary swimming supported by spine movements, tenpin-shaped habitus, rotund body cross section, ciliation of the head as two paired anterolateral tufts plus a paired posterolateral band of cilia, restriction of the muscular double helix to the mid-intestine, segmentation or partitioning of musculi laterales, presence of musculi obliqua, presence of motile trunk spines, *2* presence of dorsodermal muscle R2, (ventral ciliation consists of paired patches of cilia) (one lateral denticle per spine), *3* pharynx possesses distinct bulbs, absence of ventral trunk scales, (two lateral denticles per spine) (trunk spines of group ta are exceptionally long), *4* ciliary swimming supported by saltatory movements, inkpot-shaped habitus, motile spines strongly curved, characteristic 3-2-2-1-pattern of motile trunk spines, characteristic ventrodorsal course of all spines of group ta and first spines of groups tb and tc, spines of group td are exceptionally long and cross behind the trunk end, *5* presence of motile spines on head and neck region, presence of rear spines, *6* a single group of head plus neck spines, *7* all musculi obliqua as thin muscle strands of equal width, three groups of head plus neck spines, three trunk spines per side in group td, presence of six groups of trunk spines (ta–tf), *8* four trunk spines per side in group ta, two pairs of rear spines, *9* (ventral ciliation consists of two longitudinal rows of cilia). Abbreviations: "*Cha.*" Chaetonotidae (probably polyphyletic), *Xen.* Xenotrichulidae. The *black arrow* refers to the last common ancestor of Dasydytidae

The only useful argument for a monophyletic *Setopus* that we found is the unequal length of rear spines, a character that is also used as a traditional diagnostic feature (see emended diagnosis of *Setopus* by Kisielewski 1991). However, this single putative autapomorphy for *Setopus* is outpaced by several synapomorphic spine-related character states that argue for a *S. tongiorgii–D. goniathrix* relationship. We are sure that further muscular studies on species such as *Setopus aequatorialis*, *D.* (*Prodasydytes*) *papaveroi* or *Ornamentula paraensis* will yield important data for getting a better resolution of the internal relationships of Dasydytidae.

Discussing a possible evolutionary origin of the unique oblique musculature, Kieneke et al. (2008a) pointed to the 'multiple muscle spikes' of musculi laterales in *Halichaetonotus* sp. 1 discovered by Hochberg and Litvaitis (2003). Interestingly, the 'segments' of musculi laterales of *Dasydytes* (*Dasydytes*) *goniathrix* also form differentiations at their posterior ends comparable to the muscle spikes in *Halichaetonotus*. Since we have no certainty about the actual sister group of Dasydytidae, we are not able to conclude whether structures like these muscle spikes are putative precursors of the oblique musculature. Kisielewski (1991) suggested the *Chaetonotus* subtaxon (subgenus) *Zonochaeta* as a possible sister group of Dasydytidae. Species of *Zonochaeta* possess a traverse belt of elongated and movable spines that insert on sometimes reduced basal scales (see, e.g., Schwank 1990). It would hence be very interesting and important to investigate whether there are muscular patterns in species of *Zonochaeta* that are comparable to the special somatic musculature of Dasydytidae. The system of serially arranged motile spines and its associated musculature is doubtlessly the key feature of this monophyletic group of freshwater Gastrotricha that developed a new lifestyle and created new ecological niches. The fact that many new species and even higher-level taxa were described from tropic South America (Kisielewski 1991) may indicate that the tropical region could be the geographic center of the radiation of Dasydytidae.

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